PHASE III CLINICAL TRIAL FOR AMERICAN TEGUMENTARY LEISHMANIASIS. EQUIVALENCE BETWEEN THE STANDARD AND ALTERNATIVE SCHEME WITH MEGLUMINE ANTIMONIATE.

Subprojects

A. Controlled, randomized, double-blind, and phase III clinical trial to verify the equivalence of effectiveness and compare the safety between standard and alternative dose regimens of meglumine antimoniate in the treatment of cutaneous leishmaniasis.

B. Development and application of methodologies for analysis of antimony speciation in patients with leishmaniasis treated with meglumine antimoniate.

C. Comparison of the anti-*Leishmania* immune response in patients with American cutaneous leishmaniasis treated with standard or alternative dose of meglumine antimoniate.

D. Comparison of *in vitro* cellular immune response to reference and parasite antigens isolated from the respective American tegumentary leishmaniasis patients who evolved to cure or reactivate the lesions after antimonial therapy

E. Evaluation of genetic variability and *in vitro* antimonial sensitivity of *Leishmania (V.) braziliensis* samples isolated from patients before and after treatment with standard or alternative regimen of meglumine antimoniate.

F. Blind study to evaluate the effectiveness and safety of intralesional meglumine antimoniate in patients with cutaneous leishmaniasis and contraindication to systemic therapy.

G. Blind study to evaluate the effectiveness and safety of intralesional meglumine antimoniate in the treatment of patients with cutaneous leishmaniasis excluded from subproject A (systemic treatment with meglumine antimoniate).

H. Phase III clinical trial for mucocutaneous or mucosal leishmaniasis. Comparison between the standard and alternative scheme with meglumine antimoniate.

I. Blind study to evaluate the effectiveness and safety of intermittent low dose meglumine antimoniate in the treatment of patients with mucosal leishmaniasis excluded from the H subproject (standard or continuous low dose regimen).

J. Evaluation of patients' adherence in the Phase III clinical trial with standard and alternative scheme with meglumine antimoniate in the treatment of American cutaneous leishmaniasis.

K. Clinical-molecular study on mucosal leishmaniasis: diagnosis and screening of subpopulations of *Leishmania (Viannia) braziliensis*.

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SUBPROJECT A

A controlled, randomized, double-blind, and phase III clinical trial to verify the equivalence of effectiveness and compare the safety between standard and alternative dose regimens of meglumine antimoniate in the treatment of cutaneous leishmaniasis

A 5. Hypotheses to be tested

A 5.1 Non-inferiority of effectiveness

H_{0E} = There is no non-inferiority between the regimen currently recommended in Brazil for the treatment of cutaneous leishmaniasis (20 mg Sb5 + / kg / day for 20 days) and the alternative regimen with 5 mg for 30 days, i.e. the alternative scheme is not non-inferior to the standard regimen.

H_{1E} = the alternative 5 mg schedule is non-inferior to the currently recommended 20 mg antimony regimen.

A 5.2 Safety

H_{0S} = There is an equivalence of toxicity between the currently recommended regimen in Brazil for the treatment of cutaneous leishmaniasis (20mg Sb5 + / kg / day for 20) and the alternative regimen with 5mg for 30 days, i.e. no difference in toxicity between the schemes.

H_{1S} = the currently recommended 20 mg regimen is more toxic than the alternative regimen.

Regardless of the therapeutic regimen, lesions located above the knees are expected to be epithelialized at the end of treatment, while epithelialization of localized lesions in the legs and feet occurs more frequently after the end of the treatment period. Administration of the drugs, particularly in those cases associated with vascular insufficiency. It is also expected that, regardless of the therapeutic regimen, patients over 50 years old present adverse effects more frequently and more intensely than younger patients.

A 6. Objectives

A 6.1 General objective

To compare the efficacy and safety of meglumine antimoniate at a dose of 20 mg Sb^{5+}/ kg/day for 20 days or at 5 mg for 30 days in the treatment of patients with cutaneous leishmaniasis.

A 6.2 Specific objectives

1. To compare the immediate effectiveness (initial cure) and effectiveness after one year of follow-up (definitive cure) between the standard regimen recommended by the Brazilian Ministry of Health and the alternative regimen for the treatment of cutaneous leishmaniasis, with a non-inferiority margin of 15%.

2. To compare the frequency and severity of clinical, laboratory and electrocardiographic adverse effects among the different antimonial treatment groups.

3. Compare the frequency and severity of adverse effects and effectiveness among groups according to age, gender and race.

4. Compare the frequencies of epithelialization achieved on days 20, 30 and 50 of treatment among patient groups.

5. Compare the time in days until the epithelialization of the lesions according to the location above and below the knees, between the standard and alternative regimen of antimonial treatment, controlling for the concomitance with associated vascular insufficiency.

A 7. Subjects and methods

A 7.1 Study outline

Randomized, controlled to the standard treatment, double-blind and phase III clinical trial.

A 7.2 Description of the medication and schedule of interventions

In Brazil, meglumine antimoniate (Aventis, São Paulo, Brazil) is stored at room temperature and distributed to the health network by the Health Surveillance Secretariat

- SVS / Brazilian Ministry of Health, which will provide a single lot to be used in all patients of the study.

This drug is for intramuscular (deltoid or gluteal) application in a single daily dose. Sterile plastic disposable syringes of 5 to 20 mL and disposable, sterile 25 × 7 mm needles will be employed.

The study using the intramuscular (IM) route without direct supervision will allow the evaluation of its use in real-world conditions (effectiveness) employed by the primary health services in the state of Rio de Janeiro. Although intravenous prescription is possible, it is not a usual route of antimony administration and it is also difficult to implement in an outpatient primary setting.

Each patient will be included in one of the following meglumine antimoniate treatment groups per IM route:

1. 20mg Sb5 + / kg / day for 20 days

2. 5mg Sb5 + / kg / day for 30 days

There will be no cross-over between the groups for the purposes of this study. The data of those patients who need a definitive interruption of a regimen will be analyzed according to the group to which they were randomized, which means, by intention to treat. Data collection will take place according to the consultation schedule.

A 7.3 Sampling plan

A 7.3.1 Sample size

The groups with the minimum sample size (36 patients) are expected to respond to all the outcomes of interest.

We believe that the comparison of effectiveness between the two schemes will reveal non-inferior results to the alternative regimen for the following outcomes:

1. frequency of good initial response (evaluated on days 20, 30 and 50).

2. time (in days) until epithelization of all lesions is reached.

3. time (in days) to achieve total healing of all lesions.

4. frequency of good late response (one year of outpatient follow-up according to study schedule).

6. frequency of reactivation after treatment (up to two years of outpatient follow-up according to study schedule).

The significance level of 5% and power of 80% were used to calculate the required sample size to compare the frequencies of the outcomes of interest in the standard group with the other group. To test the non-inferiority between proportions in the primary endpoints of effectiveness, an acceptable limit of 15% difference between these healing ratios was considered. This will require 36 patients in each therapeutic regimen group.

Predicting the use of the McNemar paired test to compare the rates of healing or epithelialization on days 20, 30 and 50 will require at least 36 patients in each group (assuming a medically irrelevant difference of 30%).

To compare the effectiveness of treatment according to the location of the lesions, above or below the knee, only 12 patients will be needed in each treatment group.

It is expected that the alternative group (low dose, 5 mg Sb^{5+}/kg/day) will present non-inferior results with a margin of 15%, and a lower frequency of clinical adverse effects, in addition to a low frequency of laboratory and electrocardiographic adverse effects. However, we expect to find a significant difference in the following outcomes when comparing the safety of the standard 20mg treatment with the alternative regimen:

1. frequency of clinical, laboratory or electrocardiographic adverse effects in any degree of intensity.

2. frequency of clinical, laboratorial or electrocardiographic effects of greater intensity.

3. frequency of treatment interruption caused by Adverse Events.

4. frequency of treatment dropouts.

In the case of elderly patients, a marked increase in the frequency and severity of AE in group 1 (20mg) is expected when compared to group 2 (5mg).

The significance level of 5% and the power of 80% were used to calculate the sample size needed to compare the standard group with the other group.

A 7.3.2 Allocation strategy (randomization)

Eligible individuals (see eligibility criteria) who agree to participate (signing the informed consent form) will be allocated randomly in one of the treatment groups, according to the order of arrival, until the groups are completed. The randomized (numbered) allocation

list will be constructed in EPI-INFO 6.4 with the total number of subjects required for the survey and made available at the IPEC pharmacy. There will be stratification with interaction analysis of clinical and epidemiological aspects that may have a modifier effect on the endpoints (age, gender and race) and blocking with pre-defined blocks of twelve in order to ensure the balance between treatment groups at any time in case of need to stop the research.

A 7.4 Eligibility criteria

A 7.4.1 Inclusion criteria

1. cutaneous leishmaniasis (CL) with parasitological diagnosis by one or more of the following methods: direct examination (scraping or imprint), histopathology, culture, immunohistochemistry or PCR.

2. history of exposure in the endemic area of the state of Rio de Janeiro.

3. absence of previous treatment with meglumine antimoniate.

A 7.4.2 Exclusion Criteria

1. women who do not use contraceptive methods or do so improperly.

2. pregnant women.

3. children under 13.

4. previous treatment with meglumine antimoniate.

5. use of immunosuppressive therapy (corticosteroid, chemotherapy for cancer) or use. of medications for tuberculosis or leprosy.

6. presence of clinical baseline changes equivalent to adverse effect level> G3.

7. presence of laboratory abnormalities equivalent to adverse effect level> G2.

8. presence of baseline electrocardiographic changes equivalent to adverse effect> G4 and / or baseline QTc > 0.46ms (equivalent to G1 level A).

A 7.5 Study patients and schedule for inclusion

The study will include 72 patients with CL coming from the State of Rio de Janeiro, attended at the Reference Center on Leishmaniasis - IPEC - Fiocruz.

As lesions located below the knee are expected to only be fully epithelized several weeks after the end of treatment, bias may occur in the assessment of treatment effectiveness if patients with this lesion location were more frequently present in a given treatment group. Likewise, patients older than 50 years usually present adverse effects more frequently and with greater intensity, and may negatively influence the safety assessment if they were present more frequently in a given group. These inclusion biases will be avoided through the strategy of block randomization and controlled with multivariate analysis, considering the following variables as potential confounders: age above and below 50 years and presence of lesions above and below the knees.

A 7.6 Outcomes

A 7.6.1 Outcomes of Effectiveness: Definition

1. Initial therapeutic response - presence or absence of total epithelization of all lesions until the consultation on day 120 (initial cure).

2. Late therapeutic response - presence or absence of the following elements in the progression expected for total healing:

- disappearance of crusts until the consultation of day 140;
- disappearance of desquamation (smooth surface) until the consultation of the day 230;
- disappearance of infiltration until the consultation of day 320;
- disappearance of erythema until the consultation on day 360 (definitive cure);
- no appearance of mucosal lesion until the consultation of day 770;
- reappearance of any stage previous to that achieved, maintained in 2 observations performed with an interval of at least two weeks.

A 7.6.2 Safety outcomes (adverse events): definition, strength and relationship to study drug

An adverse event (AE) is any unanticipated or unfavorable event that either the investigator or the patient reports, starting during the use of the medication or within 30 days after its suspension. The AE examination will be made by spontaneous remembrance and questioned by the physician according to a standardized form on days 10, 20, 30, 50, 60 and 80.

The classification of the severity of adverse events (clinical, laboratory and electrocardiographic) will be given according to the tables in annexes 1 and 2 adapted from the "AIDS Table for Grading Severity of Adult Adverse Experiences, 1992" (Adult AIDS Clinical Trials Group August 1992).

The causal relationship with the study drug (= adverse effect) will be evaluated by the investigator and classified as follows:

1. Definitive (Highly Likely): A reaction that occurs within a reasonable time sequence after drug administration or when drug levels have established in body fluids and tissues; which follows a known standard response of the suspected drug; which is confirmed by the improvement after stopping the drug and reappears on repeated exposure.

2. Likely: A reaction that occurs within a reasonable time sequence after administration of the drug; which follows a known standard response of the suspected drug; which is confirmed by the improvement after stopping of the drug and which cannot be reasonably explained by the known characteristics of the individual's clinical condition.

3. Possible: A reaction that occurs within a reasonable time sequence after drug administration; following a known standard response of the suspected drug but which may be produced by the characteristics of the individual's clinical condition or other modes of therapy administered to the subject.

4. Remote (Probably Not): A reaction that occurs within a reasonable time sequence after drug administration; which follows a known standard response of the suspected drug but which can be reasonably explained by the characteristics of the individual's clinical condition.

5. Definitely No: Any reaction that does not meet the above criteria.

A 7.7 Medication allowed during the test

There will be no restrictions on the use of symptomatic medications and other diseases with the exception of those listed in the exclusion criteria (tuberculostatic, immunosuppressive and chemotherapy for cancer).

A 7.8 Handling of adverse effects

The AEs shall be recorded in an appropriate form, including: the description of the adverse effect, intensity, relationship to the investigated drug, date of onset, date of termination, duration and conduct taken.

As a general rule, appropriate measures will be taken to deal with AE described in this section.

A 7.9 Monitoring Parameters

The effectiveness and safety parameters (outcomes) will be monitored according to the implementation schedule (item A 7.7).

Ascertainment biases will be minimized through the adoption of a standardized data collection form to be completed at each consultation by the team of trained professionals. This data sheet will include adherence data to the protocol, information on the periodicity of the correct (or not) administration of the drug, the collection of biological samples for exams and the occurrence of adverse effects and outcomes of interest.

A 7.10 Monitoring adherence

Follow-up losses will be bypassed / minimized by active search: by telephone (two) and telegram (one) if no previous response is obtained. These features will be offered to all patients who miss a scheduled appointment. The patient will be asked to return the unused ampules, in order to account for the drug used.

A 7.11 Masking

It was decided that a physician who was not aware of the therapeutic scheme would perform measurements of clinical (interest) and adverse (clinical) outcomes, in order to preserve masking between intervention and outcome. This step was taken to minimize the risk of ascertainment biases originated from differentiated outcomes according to the treatment scheme to which each patient belongs. The results of laboratory tests will be provided by the clinical pathology laboratory without information on the treatment group. Similarly, for analysis purposes, groups will not be identified. The database manager will preserve the confidentiality of this information by encoding the groups for analysis by the epidemiologist(s).

A 7.12 Criteria for definitive discontinuation of study treatment

1. interruption caused by clinical, laboratory or electrocardiographic AE Grade 4

2. interruption longer than 10 days due to clinical, laboratory or electrocardiographic adverse effects Grade <3

3. spontaneous interruption of the use of prescribed medication in an amount greater than five consecutive doses due to failure of administration (non-adherence)

A 7.13 Criteria for withdrawal from the study (but not excluded from data analysis)

1. definitive discontinuation of the treatment regimen for which it was randomized for any reason;

2. pregnancy;

3. need for introduction of immunosuppressive or potentially toxic drug (chemotherapy for cancer, tuberculosis or leprosy scheme);

4. Intercurrent disease, unrelated to the drug studied, but with manifestations equivalent or superior to clinical AD Grade 3;

5. need for re-treatment due to poor initial or late therapeutic response;

6. withdrawal of the patient from continuing in the study.

All patients will receive medical care at the leishmaniasis outpatient clinic of IPEC during the occurrence of adverse events. If necessary, the medication will be suspended for a maximum of 10 days and all affected patients will receive symptomatic treatment until the complete relief of the events temporally associated with the medication. They may have their treatment continued in an alternative regimen (but for the purposes of this clinical trial they will be analyzed by intention to treat, i.e. according to the group for which they were randomized). Whenever treatment is interrupted for safety reasons for more than 10 days, the patient will be withdrawn from the trial and restarted by intralesional treatment (subproject G).

A 7.14 Procedures for breach of confidentiality

Randomization codes generated by software and used in the allocation of numbers and allocation of patients may be unraveled in case of extreme need and always considering

the well-being of the patient. For this purpose, a copy of the randomization scheme will be in possession of an epidemiologist physician not connected to the patient and / or data analysis team, who may be contacted at any time to clarify in case of emergency the type of dose and schedule which the patient belongs.

A 7.15 Study monitoring

A 7.15.1 Coordinators and Field Monitor

The main investigator and coordinators will oversee the fieldwork, controlling for quality and protocol deviations. Important items to monitor are: adequate completion of outcome records and adverse events; adequacy of stored medicines; quality of laboratory examination procedures; minimization of missing data; periodically sending data for typing. Written field reports will be kept for consideration by committees. The main investigator and coordinators will also be in charge of reporting on any serious adverse events to the Research Ethics Committee / IPEC and deciding when to interrupt the trial.

A 7.15.2 External Committee

An external monitoring committee will be constituted in this trial consisting of three expert members in the treatment of leishmaniasis and the execution of clinical trials. Members will be chosen from curriculum Lattes database according to the appropriateness of their function- and competence-based profiles. The committee will audit the documentation and activities pertinent to the clinical trial, assessing possible deviations from the protocol.

A 7.16 Control of dispensing and storage of medications

All ampoules needed for the complete treatment of the entire study population will be stored at the IPEC pharmacy. A trained team professional will recruit the patients at the day 1 appointment, following the randomization list from the trial previously provided by the Epidemiology sector. A trained pharmacist will dispense the medication prescribed by the Infectious Diseases expert physician by submitting the test card and the prescription.

A 7.17 Data Analysis Plan

Data analysis will be performed following the intent-to-treat principle, supplemented by a per-protocol analysis of the primary endpoint. Data from those patients who need a definitive interruption of treatment will be analyzed according to the group to which they were initially allocated, and will not be reassigned in another group to resume treatment (there will be no cross-over between the groups for the purposes of this study). The non-inferiority hypothesis will be tested based on the non-inferiority margin of 15% and a one-sided confidence interval of 95%.

Simple frequencies of categorical variables (gender, race, location of lesions, co-morbidity, adverse events, treatment completion or non-recurrence, relapse) and measures of central tendency and dispersion of continuous quantitative variables (age, number of lesions; time to treatment in days, time to reach the initial and late effectiveness outcomes) for each antimonial scheme used (20 mg or 5 mg) will be described in this study.

Healing frequencies will be compared through the chi-square test, the mean time to healing through a three-way or more (ANOVA) means-comparison test and survival analysis for time-related outcomes in days, non-parametric tests will be used if necessary. To assess effectiveness and safety, relative risk (RR), as well as absolute risk reduction (ARR) and relative risk reduction (RRR) will also be estimated.

For the matched comparison of healing rates on days 20, 30 and 50, the Mann-Whitney test will be used.

A 8. Ethical considerations

A 8.1 Risks and benefits

The main potential benefit of this trial is the possibility of subsidizing the use of lower, potentially less toxic and lower cost antimony doses for the treatment of cutaneous leishmaniasis that affects a large number of Brazilians, including elderly patients with comorbidities (heart, kidney and liver diseases). Risks consist of general adverse effects, which will be carefully scrutinized and treated in accordance with the attached schedule. This project will be submitted to CEP / IPEC and CONEP. All patients will sign an informed consent form approved by CEP / IPEC. This project follows the recommendations contained in resolution 196/96 of the National Health Council.

A 8.2 Term of Free and Informed Consent

In accessible language and clarifying objectives, risk, benefits and identifying those responsible for the research.

A 8.3 Incentives for volunteers

Volunteers will receive transportation assistance and medications.

A 9. Expected Results

It is expected that the alternative regimen with 5mg for 30 days will be non-inferior in effectiveness to the scheme currently recommended in Brazil for the treatment of cutaneous leishmaniasis (20mg Sb5 + / kg / day for 20 days).

However, a significant difference in the toxicity of the different schedules is expected, which should show adverse effects (in frequency and intensity) in the following decreasing order: 1) 20mg Sb5 + / kg / day for 20 days; 2) 5mg Sb5 + / kg / day for 30 days.

Regardless of the therapeutic scheme, lesions located above the knees are expected to be epithelialized at the end of treatment, while the epithelization of localized lesions in the legs and feet occurs more frequently after the end of the administration period. Particularly in those cases with associated vascular insufficiency. It is also expected that, regardless of the therapeutic regimen, patients over 50 years old present adverse effects more frequently and more intensely than younger patients.

The results of this project should be published in indexed journals and in scientific events in the areas of parasitology, molecular biology, infectious diseases and tropical medicine. The coordinators and some researchers will be responsible for organizing the manuscript and communicating the results.

A 10. Financial support

This project is partially funded with resources approved by the Edict MCT / CNPq / MS-SCTIE-DECIT 25/2006 - Neglected Diseases Study - and will be submitted to other relevant edicts of the development agencies.

A 11. Foreign cooperation, storage of biological samples and intellectual property

In this project, there will be no cooperation with foreign entities nor storage of biological samples. There is also no expectation of any patent applications for products and procedures.

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ATTACHMENT

Free and informed consent form

INSTITUTION: Evandro Chagas Clinical Research Institute - Fiocruz

RESEARCH COORDINATOR: Armando de Oliveira Schubach

ADDRESS: Av. Brasil 4365 - Manguinhos - Rio de Janeiro - RJ - CEP 21040-900

TELEPHONES: (0xx21) 3865-9525 / 3865-9541 / FAX (0xx21) 3865-9541

NAME OF THE RESEARCH PROJECT:

Phase III clinical trial for American Cutaneous Leishmaniasis. Equivalence between the standard and alternative scheme with meglumine antimoniate

NAME OF VOLUNTEER: _____

This document seeks to clarify the health problem under study and the research that will be performed, providing information, detailing the procedures and examinations, benefits, drawbacks and potential risks.

American tegumentary leishmaniasis (ATL) is a disease caused by parasites called *Leishmania* and presents as wounds on the skin that is difficult to heal. Sometimes the ATL may become more severe, involving the lining of the nose and throat, even several years after the healing of the wound on the skin. Currently, we cannot quite predict which patient will fall ill again and which will remain permanently cured.

In Brazil, the Ministry of Health (MS) recommends treating patients with ATL with meglumine antimoniate in high doses (20mg per kilogram of body weight per day) for 20 to 30 days, respecting the maximum limit of 3 daily ampoules. However, changes in the

kidneys, heart, liver, pancreas and blood tests are frequent. In addition to joint pain and discomfort at the site of intramuscular injections.

In the IPEC Reference Center on Leishmaniasis, Fiocruz, a low dose of meglumine antimoniate (5 mg per kilogram of body weight per day) has been shown to be effective and well tolerated in the treatment of patients with ATL. Patients with cutaneous form are treated for 30 days. Patients with mucosal form are treated continuously for a minimum of 30 days, preferably without interruption, until mucosal healing, which usually occurs between 30 and 90 days of treatment. Elderly patients or other associated diseases are treated with low doses in series of 10 days at 10 day intervals without medication. Patients who are contraindicated for intramuscular treatment or who show signs of intoxication during treatment may be treated with one or two applications of meglumine antimoniate directly into the skin lesion.

Our accumulated experience suggests that alternative treatment regimens have the same good results as the standard regimen recommended by the Brazilian Ministry of Health, but with fewer adverse effects. However, it is only after the conclusion of this study that we can suggest that the Brazilian Ministry of Health should change recommendations for the treatment of ATL.

Now that your LTA diagnosis has been confirmed, you are being invited to participate in a clinical investigation to be conducted at IPEC-Fiocruz, with the following objectives:

- To evaluate the response to the treatment of ATL with the use of different doses or forms of application of antimonials.
- Describe the behavior of antimonials in the human body according to the different treatment schemes.
- To compare the immunological response of patients treated with different regimens.
- Characterize the isolates of *Leishmania* and check sensitivity to antimonial.

Your participation in this study is entirely voluntary. You may refuse to participate in one or all stages of the research, or even withdraw from it at any time, without this fact causing you any embarrassment or penalty on the part of the Institution. Your medical care will not be affected if you decide not to participate or if you decide to leave the study already started. Your doctors may also interrupt your participation at any time if they deem it convenient for your health.

Their participation in relation to the project is to authorize the indication of their treatment for cutaneous form of ATL with meglumine antimoniate intramuscularly, be done by lot

for one of the following groups: 1) high dose for 20 continuous days; 2) low dose for 30 continuous days. If there is any contraindication for you to receive any of these regimens or intolerance in need of interrupting a started regimen, treatment will be performed with one or two medication applications, with a two-week interval, directly on the skin lesion. In the case of a mucosal form you can be drawn to one of the following groups: 1) high dose for 30 days; 2) low dose daily until curing. If there is any intolerance with the need to interrupt one of the initiated regimens, the treatment will be performed at low dose in series of 10 days with intervals of rest, then until the cure. Doctors who will evaluate your treatment will not know which regimen is used and you will not know if you are being treated at high or low dose so as not to be influenced in the trial.

Your authorization will also be required: 1) for the use of photographic documentation or filming of your lesions for study; 2) so that part of the material collected periodically to perform examinations to monitor the evolution of their disease, as well as the results of these routine exams and their treatment are used in this study; 3) so that part of the collected samples are stored in order to serve other studies aimed at better understanding the disease, the development and evaluation of new diagnostic methods; evaluation of treatment response; and so on, provided that such study is previously analyzed and authorized by a Research Ethics Committee.

By participating in this study, you will have some responsibilities: follow your doctor's instructions; attend the health facility on the scheduled dates; and report to your physician all reactions you present during treatment, both positive and negative. The examinations and procedures applied will be free of charge. You will receive all appropriate medical care for your illness. If you need medical care, during the period you are participating in the study, even outside your appointment, contact the Evandro Chagas Clinical Research Institute - Fiocruz. In case of need, call Dr. Armando de Oliveira Schubach, Dr. Cláudia Maria Valete Rosalino or Dr. Maria Inês Pimentel in the above telephones. If you have any problem that requires hospitalization, the medical team will provide your bed at the Evandro Chagas Clinical Research Institute - Fiocruz.

Your identity will be kept confidential. The results of the study may be published without revealing your identity and your images may be released as long as you cannot be recognized. However, if necessary, your medical records will be available for consultation to the team involved in the study, to the Research Ethics Committee, to the Health Authorities and to you.

You can and should ask any questions you deem necessary before agreeing to participate in the study, as well as at any time during treatment. Your doctor should

provide all necessary information regarding your health, your rights, and any risks and benefits related to your participation in this study.

Disadvantages and main risks known to date: meglumine antimony agent usually causes undesirable effects, should not be used during pregnancy and its use in women of reproductive age should be accompanied by the use of an effective contraceptive method as a male or female latex condom ("Condom"), female diaphragm or oral contraceptive ("pill").

Forms of reimbursement: When necessary, on the days of their attendance, food may be provided according to routine of the Nutrition and Social Service of IPEC for outpatients.

Expected benefits: You are expected to be cured of the ATL at the end of treatment, although return visits for several years after treatment are necessary to confirm cure. The results of this study may not benefit you directly, but, in the future, may benefit others, as this study is expected to contribute to a better monitoring of treatment of patients with ATL, which may be done more effectively and safely.

I declare that I have read and understood all the information regarding this study and that all my questions have been adequately answered by the medical staff, who will be on hand to answer my questions whenever I have questions.

I have received a copy of this consent form and hereby consented voluntarily to participate in this research study.

_____ _____

Patient Name: Date

_____ _____

Medical Name: Date

ANNEX (TERM OF CONSENT FOR DIAGNOSTIC PROCEDURES)

Free and informed consent form

INSTITUTION: Evandro Chagas Clinical Research Institute - Fiocruz

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NAME OF THE RESEARCH PROJECT:

Study for the systematization of the care of patients with American Cutaneous Leishmaniasis in the Reference Center in ATL - Evandro Chagas Clinical Research Institute - Fiocruz

NAME OF VOLUNTEER: _____

American tegumentary leishmaniasis (ATL) is a disease that strikes humans and animals, including the dog, caused by parasites called *Leishmania*. The disease is transmitted by the "straw mosquito", which lives in areas of forest, banana plantations, mango etc and are located near human dwellings, where they usually enter to feed on the blood of people and domestic animals. The ATL presents as hard-to-heal wounds on the skin. Sometimes the ATL may become more severe, involving the lining of the nose and throat, even several years after the healing of the wound on the skin. Currently, we cannot quite predict which patient will fall ill again and which will remain permanently cured.

Other diseases such as bacterial infections, tuberculosis, syphilis, sporotrichosis, other mycoses, tumors etc can manifest in a manner similar to leishmaniasis and need to be differentiated in order to initiate the correct treatment. However, with the exams currently available, you cannot always be absolutely certain about the disease in question.

At the moment, several questions need to be answered like: In what other ways can ATL manifest itself? How do laboratory tests behave before, during and after treatment? Which patients, even after treatment, will reopen their scars or develop disease inside the nose or throat? Which other similar diseases are being confused with the ATL and which exams should be used for clarification? What is the role of humans as reservoirs

of disease? What are the best forms of treatment? What steps should be taken to control the problem?

You are being invited to participate in a clinical investigation to be held at IPEC-Fiocruz, with the following objectives:

- Describe aspects of ATL: clinical manifestations and laboratory tests, trying to establish patterns of presentation of the disease and its mode of evolution, comparing with other diseases.
- To evaluate the use of antimonials and other drugs used in the treatment of ATL, taking into consideration treatment time, toxicity, ease of administration, cost and absence of involvement of the mucous membranes of the nose and throat.
- Isolate, identify and compare ATL-causing *Leishmania* parasites from several localities.

This document seeks to clarify the health problem under study and the research that will be performed, providing information, detailing the procedures and examinations, benefits, drawbacks and potential risks.

Your participation in this study is voluntary. You may refuse to participate in one or all stages of the research, or even withdraw from it at any time, without this fact causing you any embarrassment or penalty on the part of the Institution. Your medical care will not be affected if you decide not to participate or if you decide to leave the study already started. Your doctors may also interrupt your participation at any time if they deem it convenient for your health.

Your participation in relation to the Project is to authorize a series of tests to diagnose our illness, and part of this material, as well as the results of these routine exams, are used in this study. Your authorization will also be required: 1) for the use of photographic documentation or filming of your lesions for study, 2) so that part of the material collected periodically to perform tests to monitor the evolution of your disease, as well as the results of these routine tests and its treatment are used in this study, 3) so that part of the collected samples is stored in order to serve other studies aimed at better understanding the disease, the development and evaluation of new diagnostic methods; evaluation of response to treatment etc., provided that such study is previously analyzed and authorized by a Research Ethics Committee.

The examinations and any procedures applied will be free of charge. You will receive all appropriate medical care for your illness.

By participating in this study you will have some responsibilities: strictly follow your doctor's instructions; attend the health facility on the scheduled dates; tell your doctor all the reactions you present during treatment, both positive and negative. If you need medical attention, during the period in which you are participating in the study, go to the Evandro Chagas Clinical Research Institute - Fiocruz, even outside your appointment day. In case of need, please call Dr. Armando de Oliveira Schubach, Dr. Fátima Conceição-Silva or Dr. Mariza Salgueiro on the above telephones. If you present any clinical condition that requires hospitalization, the medical team will provide your hospital bed at the Evandro Chagas - Fiocruz Clinical Research Institute. Your animals with suspected LTA may be treated free of charge by veterinarian Dr. Tânia Maria Valente Pacheco at the IPEC Zoonoses Service.

Your identity will be kept confidential. The results of the study may be published without revealing your identity and your images may be released as long as you cannot be recognized. However, if necessary, your medical records will be available for consultation to the team involved in the study, to the Research Ethics Committee, to the Health Authorities and to you.

You can and should ask any questions you deem necessary before agreeing to participate in the study, as well as at any time during treatment. Your doctor should provide all necessary information regarding your health, your rights, and any risks and benefits related to your participation in this study.

Procedures, exams and tests to be used:

Before treatment there will be collection of information about the disease; general medical examination and examination of the skin with description and photographic documentation or filming of the lesions; Internal examination of the nose and throat with a device called optical fiber, which allows viewing of small lesions or difficult to reach sites for description and photographic documentation or filming of the lesions (local anesthetic spray if necessary). Removal with local anesthesia of a small fragment of the skin, mucosal or "inguinal" lesion to perform diagnostic tests (microscopic appearance of diseased tissue and cultures to try to isolate possible disease agents such as fungi, bacteria and *Leishmania* parasites) and for research (identification of cells and other components of the inflammatory response, as well as new methods for identifying possible agents of the disease). Other materials may also be collected in an attempt to isolate the causative agent: syringe and needle aspiration from the edge of the lesion and secretions from closed skin lesions.

Other tests will also be done to diagnose other diseases that may be confused with ATL, to classify disease severity, and to evaluate the effects of medications to be used during your treatment: one to four skin tests (one-tenth of a milliliter reaction for a certain disease in the skin of the anterior region of the forearm, which should be reviewed between 2 and 3 days after the injection); Blood samples (equivalent to approximately three tablespoons), saliva (collected with a type of swab), radiography of the lungs and face (if necessary supplemented by computed tomography); and electrocardiogram.

The treatment of ATL in human patients is usually with intramuscular (IM), intravenous (IV) meglumine antimonia agent an injection a day, usually for a continuous period of 30 days or with rest intervals. Exceptionally, for the elderly, patients with severe diseases or who do not tolerate normal treatment, the intralesional route (IL) may be used. Treatment time may be shortened or increased as needed. Other treatment options are amphotericin B (IV) and pentamidine (IM), both injectables and requiring follow-up measures similar to those of meglumine antimoniate.

After initiation of treatment, you should attend approximately three appointments within 10, 20, and 30 days. If the lesions do not heal completely, treatment may be continued for as long as necessary. Upon clinical cure, you should return for reassessment at 1, 3, 6, 9, and 12 months after the end of treatment. And thereafter, at least once a year for an indefinite period (at least 5 years).

Medical evaluation and blood tests should be performed at each evaluation day (in the approximate amount of one or two tablespoons) to evaluate the effects of the medicines used in their treatment and / or to evaluate the evolution of the disease. Other tests, such as the electrocardiogram during treatment, may be performed when indicated.

Main drawbacks and risks known to date:

The collection of blood may cause some pain at the time of venipuncture and, eventually, there may be a purple area on the site, which will return to normal within a few days.

Occasionally, skin tests may show a strong reaction with local inflammation, blistering, and, more rarely, wound formation. The whole process usually recedes within a few days to a few weeks.

Both skin tests and the anesthetic injected at the time of biopsy (removal of a small piece of skin for examination) may cause allergy, usually limited to the appearance of red, itchy, itchy areas on the skin and respond well to anti-allergic drugs. More rarely, there may be a more severe reaction to breathing and the need for a more intensive care in IPEC.

At the site of the biopsy, inflammation and pain may occur, with or without bacterial infection. If this occurs, you may need to use pain medications and antibiotics. Medications such as meglumine antimoniate and pentamidine usually cause undesirable effects, should not be used in pregnancy and their use in women of reproductive age should be accompanied by the use of effective contraceptive method as a condom for male or female latex (female condom), female diaphragm or oral contraceptive ("pill"). When treatment cannot be delayed, amphotericin B may be used during pregnancy. X-rays should also not be performed on pregnant women.
Forms of compensation:

When necessary, on the days of its attendance, food may be provided according to routine of the Nutrition and Social Service of IPEC for outpatients.
Expected benefits:

It is expected that at the end of the treatment you will be cured of ATL, although return visits for several years after treatment are necessary to confirm cure. The results of this study may or may not benefit you directly, but in the future, it may benefit others, as it is expected that this study will contribute to a better diagnosis and follow-up of patients with ACL, and may provide a basis for the treatment to be done more effectively and safely.

If your research demonstrates a different diagnosis than ACL, you will be properly advised to seek the most appropriate treatment for your case.
I declare that I have read and understood all the information regarding this study and that all my questions have been adequately answered by the medical staff, who will be on hand to answer my questions whenever I have questions.
I have received a copy of this consent form and hereby consented voluntarily to participate in this research study.

Patient	Name:	Date

Medical	Name:	Date
Witness[1]	Name:	Date
Witness name[2]:		Date

Research Ethics Committee (REC)

CONSOLIDATED AUDIT OPINION

Protocol 0055.0.009.000-07

1. Identification:

Project Title: "Phase III clinical trial for American tegumentary leishmaniasis. Equivalence between the standard and alternative scheme with meglumine antimoniate ".

Subproject A: "Controlled, randomized, double-blind, and phase III clinical trial to verify the effectiveness and compare the safety between standard and alternative dose regimens of meglumine antimoniate in the treatment of cutaneous leishmaniasis"

Researcher in charge: Armando de Oliveira Schubach.

Institution in charge: Evandro Chagas Institute of Clinical Research / Fiocruz.

Date Submitted to the REC: 25/09/2007.

In addition to the Consolidated Audit Opinion dated from October 17th, 2007, we would like to inform that in 2008 there were additional information about some changes related to the Subproject A:

1) Modification of the clinical trial of equivalence to a non-inferiority trial with a non-inferiority margin of 15%.
2) Number of patients needed to be included in this subproject will be 72 patients in total.
3) Patients will be recruited in two groups of treatment: 20 mg Sb5+/kg/day for 20 days and 5 mg Sb5+/kg/day for 30 days.

In this sense, we would like to clarify that on the date of the first Consolidated Audit Opinion, this addition was not previously and clearly informed.

For this reason, we would like to request that from this date onwards that the new information should be incorporated into the final modification of the aforementioned Consolidated Audit Opinion.

The Consolidated Audit Opinion of 2008 has the following connotation:

Signature of coordinator:

Dra. Lea Ferreira Camillo-Coura

Research Ethics Committee Coordinator

IPEC – FIOCRUZ

Research Ethics Committee (REC)

CONSOLIDATED AUDIT OPINION

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Institution in charge: Evandro Chagas Institute of Clinical Research / Fiocruz.

Date Submitted to the REC: 25/09/2007.

2. Summary:

This constitutes an amendment to Subproject A of the main project, with modification of the equivalence study for a non-inferiority study with a margin of 15%.

This subproject is a phase III randomized controlled clinical trial in 72 patients with cutaneous Leishmaniasis (LC) treated at the Reference Center on Leishmaniasis - IPEC / Fiocruz. Eligible individuals who agree to participate will be allocated randomly in one of two treatment groups: 20 mg Sb5 + / kg / day for 20 days and 5 mg Sb5 + / kg / day for 30 days. It has as main objective to compare the schemes with meglumine antimoniate recommended in Brazil for American Tegumentary Leishmaniasis (ACL) with the alternative scheme. Specific objectives: to compare effectiveness with a 15% non-inferiority margin and safety between the groups in the treatment of cutaneous leishmaniasis (CL). The main potential benefit of this trial is the possibility of subsidizing the use of lower, potentially less toxic and lower cost antimony doses for the treatment of cutaneous leishmaniasis, including elderly patients with comorbidities (heart, kidney and liver diseases).

3. General Comments: (In compliance with Resolution CNS 196/96).

The research project has an adequate design plan. The term of free and informed consent was prepared in a language accessible to the research subject. This project is partially funded with resources approved by edict MCT / CNPq / MS-SCTIE-DECIT 25/2006.

4. Diligences:

Yes. They were satisfied.

5. Opinion: APPROVED.

Date: September 1st, 2008

Signature of coordinator:

Dr. Lea Ferreira Camillo-Coura

Research Ethics Committee Coordinator

IPEC - FIOCRUZ